only the sum of the rate coefficients can be measured for a two state system, maximum likelihood methods allow the determination of the individual rate coefficients, and therefore also the equilibrium constant. Here we apply a maximum likelihood method recently developed by Gopich and Szabo (*J.Phys.Chem.*113pp10965-10973(2009)) to the photon-by-photon trajectories of a two-state designed protein, α_3D, that folds in ~ 1ms, too fast to measure accurately from waiting time distributions in FRET trajectories. Experiments were carried out on both freely-diffusing and immobilized molecules. The FRET efficiency distributions in the free diffusion experiment are broadened by the folding/unfolding transitions occurring within the time bin, a phenomenon similar to line broadening in NMR experiments. Mean FRET efficiencies and rate coefficients extracted using the Gopich-Szabo method were found to be reliable by comparing the sum of the rates with the relaxation rates obtained from the donor-acceptor cross correlation function. Finally, photon trajectories can be divided into folded and unfolded segments at a single photon level using the hidden Markov model (Viterbi algorithm) with extracted parameters.

158-Pos

Conformations and Dynamics of Polypeptide Chains Revealed By Tryptophan-Cysteine Contact Formation Kinetics

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Spectroscopic probes sensitive to intra-chain contact formation events in polypeptides are increasingly used to study the conformational and dynamical properties of different amino acid sequences. Quenching of the triplet state of tryptophan by close contact with cysteine enables the measure of contact formation rates without the need of extrinsic probes, thus being suitable for the study of natural proteins and peptides. We illustrate the use of this method to investigate the unfolded state of small proteins in conditions close to native and the kinetics of weakly structured protein fragments. The coexistence of different conformational states can be revealed from the non-exponential relaxation of the excited triplet, enabling the characterization of both the chain dynamics for each state and the transition kinetics. Moreover, the rate of contact formation measured for the least structured states is compared with those observed for model disordered peptides, allowing to estimate the strength of electrostatic and hydrophobic interactions between residues other than the probes. We test this approach with the widely studied GB1 15-residue C-terminal, which folds into a beta-hairpin structure. The kinetics of elementary conformational steps leading to the folded state is outlined, revealing the presence of misfolded states as proposed in recent computational works.

159-Pos

Electrostatic Interactions Affect the Mechanical Stability of Elastomeric Proteins

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It was predicted that the mechanical stability of elastomeric proteins can be affected by electrostatic interactions (Craig et al, Structure, 12, 2004, 21). To directly test this prediction, we engineered a bi-histidine mutant of a small protein GB1 (biHis-GB1). The two histidines were engineered across two force-bearing beta strands. Histidine residues can exist as protonated or deprotonated states depending on pH, thus we can adjust the pH value of the solution to modulate the electrostatic interactions between the two engineered histidine residues. We used single molecule atomic force microscopy to directly measure the effect of electrostatic interactions on the mechanical stability of biHis-GB1. We found that the unfolding force of biHis-GB1 gradually decreases as the electrostatic repulsion increases due to the lowering of pH value from 8.5 to 4. This result suggested that electrostatic interactions can indeed affect the mechanical resistance of biHis-GB1. We anticipate that this effect can be utilized as an effective method to tune the mechanical stability of elastomeric proteins at the single molecule level.

160-Pos

Molecular Mechanism of Urea-Induced Protein Denaturation

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For more than half a century, urea has been used as a strong denaturant in protein folding/unfolding studies. However, the molecular mechanisms of urea-induced protein unfolding still remain unclear. This lack of understanding is to some extent reflects the scarcity of direct thermodynamic information that can be used to characterize interactions of urea with amino acid side chains and the peptide group. We recently demonstrated that volumetric measurements combined with statistical thermodynamic approach may represent a novel and effective way to tackle this problem [Lee, S. & Chalikian, T. V. (2009) J. Phys. Chem. B. 113, 2443-2450]. In this work, we employ high precision acoustic and densimetric techniques to quantify the solvation properties of solutes in the presence of urea. Specifically, we report the partial molar volumes, V°, and adiabatic compressibilities, K_S°, of *N*-acetyl amino acid amides containing all 20 naturally existing amino acid side chains and oligoglycines, $(Gly)_{1\text{-}5}$, at urea concentrations ranging from 0 to 8 M. Using our developed statistical thermodynamic approach, that links volumetric observables of a solute with solute-solvent and solute-cosolvent interactions in binary solvents, we evaluate the binding constants, k, and elementary changes in volume, ΔV, and compressibility, ΔK_S, accompanying the replacement of water in the vicinity of the solutes with a urea molecule. While the binding constants are essentially similar for all protein groups, the magnitude and the sign of the determined values of ΔV and ΔK_S vary markedly. The latter values reflect the nature of urea interactions with specific functional groups and the concomitant changes in hydration. In general, our results are consistent with a picture in which urea interacts with polar, non-polar and charged groups with comparable affinities, although the underlying forces stabilizing each type of interaction depend on the chemical nature of the interacting group.

161-Pos

Confined Dynamics of a Ribosome-Bound Nascent Globin: Cone Angle Analysis of Fluorescence Depolarization Decays in the Presence of Two Local Motions

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We still know very little about how proteins achieve their native three-dimensional structure in vitro and in the cell. Folding studies as proteins emerge from the mega Dalton-sized ribosome pose special challenges due to the large size and complicated nature of the ribosome-nascent chain complex. This work introduces a combination of three-component analysis of fluorescence depolarization decays (including the presence of two local motions) and in-cone analysis of diffusive local dynamics to investigate the spatial constraints experienced by a protein emerging from the ribosomal tunnel. We focus on E. coli ribosomes and an all-alpha-helical nascent globin in the presence and absence of the chaperones DnaK and trigger factor. The data provide insights on the dynamic nature and structural plasticity of ribosome-nascent chain complexes. We find that the sub-ns motions of the N-terminal fluorophore, reporting on the globin dynamics close to the N terminus, are highly constrained both inside and outside the ribosomal tunnel, resulting in high-order parameters ($>$0.85) and small cone semiangles ($<30^\circ$). The shorter globin chains buried inside the tunnel are less spatially constrained than those of a reference sequence from a natively unfolded protein, suggesting either that the two nascent chain sequences have a different secondary structure and therefore sample different regions of the tunnel or that the tunnel undergoes local structural adjustments to accommodate the globin sequence. Longer globins emerging out of the ribosomal tunnel are also found to have highly spatially constrained slow (ns) motions. There are no observable spectroscopic changes in the absence of bound chaperones. The data presented here show that the ribosome plays an active role in cotranslational folding and it influences the dynamics and conformation, of nascent polypeptides and proteins.

162-Pos

Unraveling the Possible Mechanism Behind Leptomeningeal Amyloidosis Using as Model a Highly Unstable Transthyretin Tetramer

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Transthyretin (TTR) is a 127-residue -βsheet-rich protein homotetrameric that transports thyroxine in the blood and cerebrospinal fluid (CSF). Among all TTR variants, A25T is the most unstable tetramer. Its great instability induces TTR degradation in the endoplasmic reticulum of the hepatocytes, while thyroxine (T4, a natural ligand of TTR) leads to A25T secretion in the CSF by the choroid plexus. In the present study we aimed to determine the structure of A25T by X-ray crystallography in the apo form and in complex with T4. Also, by using high hydrostatic pressure, we have showed that the tetramers of A25T were less stable than the wt and L55P (the most aggressive variant of TTR). Besides, A25T showed to be the most amyloidogenic variant thus far investigated, aggregating in conditions where wt and L55P remain mostly soluble. Using HPLC and native PAGE, we monitored acrylodan-labeled TTR aggregation in the human plasma. The aggregates formed displayed the typical amyloid structure. In the presence of monomers of T119M, a non-amyloidogenic variant, aggregation of A25T was remarkably reduced, pointing to the use of T119M monomers as a strategy to avoid TTR aggregation. The crystal structure of A25T, when compared to that of the wt protein, shaded light into the

mechanism behind its increased amyloidogenicity: an expanded tetramer which is stabilized by a lower number of H-bonds and hydrophobic interactions. Interestingly, in the presence of T4 and lumiracoxib the structure of A25T was similar to that displayed by the wt protein. These data show that an expanded A25T tetramer with a decreased thermodynamic stability is prone to aggregate forming amyloid fibrils that trigger leptomeningeal amyloidosis. Support: CNPq and FAPERJ.

163-Pos

Thermal Stability of the Extracellular Hemoglobin of *glossoscolex Paulistus*: Differential Scanning Calorimetry (dsc) and Circular Dichroism (cd) Studies

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Hemoglobin of *Glossoscolex paulistus* (HbGp) in the oxy- and cyanomet-forms was studied by circular dichroism (CD) and differential scanning calorimetry (DSC). DSC experiments were performed for protein concentration 0.5 mg/ml and scan rate of 1°C/min, at pH 7.0. CD experiments were performed in the near UV to monitor the peptide region (0.2 mg/ml of protein, 195-250 nm) as well as in the Soret band spectral region (3.0 mg/ml protein, 500-250 nm) to monitor changes in the heme group envinronment, in the pH range 5.0-9.0. Experiments were made in the range 25-70°C. Analysis of CD data, based on a two-state thermodynamic denaturation model, allowed to obtain the fraction of denatured protein, critical temperatures as a function of pH, equilibrium constants and corresponding free energies. Cyanomet-HbGp (Tm=65°C at pH 7.0) is significantly more stable as compared to the oxy-form (Tm=59°C). Our CD data suggests that the protein denaturates as a whole, loosing its secondary structure simultaneously for all domains of the oligomer. Critical temperatures are smaller as the pH increases in the alkaline range. On the other hand, DSC results suggest that the denaturation for oxy-HbGp is more complex, presenting low cooperativity since the endotherm could be fitted only for two components centered at 58.3 $\pm$ 0.2 and 60.6 $\pm$ 0.1°C. For the cyanomet-form the best fit for the endotherm corresponds to three components centered at 61.6 $\pm$ 0.2, 64.8 $\pm$ 0.2, and 67.2 $\pm$ 0.2°C. DSC data, in agreement with CD, also support the higher thermal stability of cyanomet-HbGp as compared to the oxy-form. Support: FAPESP, CNPq and CAPES Brazilian agencies.

164-Pos

On the Thermal Stability of Extracellular Hemoglobin of *glossoscolex Paulistus*: Optical Spectroscopic Studies

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Hemoglobin of *Glossoscolex paulistus* (HbGp) in the oxy- and cyanomet-forms was studied by dynamic light scattering (DLS) and optical absorption spectroscopy (OAS). At 25°C, oxy-HbGp, in the pH range 5.0-8.0, is stable presenting a mono-disperse size distribution with hydrodynamic diameter (D_h) of 27 $\pm$ 1 nm. Cyanomet-HbGp behaves in a similar way up to pH 9.0. More alkaline pH, above 9.0, induced an irreversible dissociation process, resulting in smaller D_h of 10 $\pm$ 1 nm, suggesting oligomeric dissociation. At pH 7.0, no oligomeric dissociation is observed as a function of temperature and denaturation occurs at 52°C and 57°C, respectively, for oxy- and cyanomet-HbGp. Dissociation temperatures were lower at higher pH, for both forms of HbGp. Based on the higher critical denaturation and dissociation temperatures cyanomet-HbGp is more stable than the oxy- form. Kinetic studies were performed for oxy-HbGp using UV-VIS OAS and DLS. Rate constants as a function of temperature and the activation energy (E_a) have been estimated by DLS for oxy-HbGp at pH 7.5 and 8.0, giving E_a values of 278 and 262 kJ/mol, respectively. Auto-oxidation kinetics monitored by UV-VIS at pH 8.0 in the temperature range 38-44°C is mono-exponential with an E_a value of 333 kJ/mol. Oligomeric protein dissociation promotes an increase in auto-oxidation rate and vice-versa. The present work shows that DLS is suitable to follow quantitatively the changes on the oligomerization of multisubunit proteins. Support: FAPESP, CNPq and CAPES Brazilian and FCT-MCTES Portuguese agencies.

165-Pos

Geometry and Efficacy of Trp-Trp, Trp-Tyr and Tyr-Tyr Aromatic Interaction in Cross-Strand Positions of a Designed β-Hairpin

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Analysis of the impact of neighboring aromatic groups on structure can lead to improved understanding of protein folding mechanisms and stability. In this study, we examined the impact of varying aromatic interactions in cross-strand positions for Trpzip2, a β-hairpin forming peptide (Cochran et al., PNAS, 2001, 98, 5578-5583), by comparison of the interactions of Trp-Trp, Trp-Tyr and Tyr-Tyr. NMR and optical spectra (ECD, FTIR) of the original TZ2 peptide and its Tyr and Val-substituted mutants were analyzed to characterize their conformations and thermal stability. Cross-strand coupled Trp-Trp and Trp-Tyr pairs show unique, strong exciton bands in ECD while the Tyr-Tyr pair doesn't show any clear exciton band. The edge-to-face cross strand interaction leads to stable β-hairpin structures when Trps are at positions 2-11 or 4-9, but the alternate coupling for Trp at positions 2-9 does not lead to a stable structure. In Trpzip2 these would correspond to a more face-to-face interaction, which may contribute to the instability. When Tyr is substituted for Trp, the Trp-Trp interaction has more contribution to the peptide stabilization than does the Tyr-Tyr pair. When Tyr is substituted into position 4 and 11, the Trp-Tyr pair also has a unique geometry. These aromatic-aromatic interactions were also compared to simple hydrophobic interaction by contrasting stabilities of peptides with Val or Tyr substituted for two interacting Trp residues. Tyr is more stabilizing than Val for such substitutions which may indicate coupling of conjugated π-electron systems dominates stability. Aromatic interactions showed a stronger effect than hydrophobic interaction for stabilization. Extended kinetic studies using laser initiated T-jumps and IR detected conformational changes have helped sort out mechanistic aspects of this folding problem (Hauser et al, JACS, 2008, 130, 2984-2992).

166-Pos

Effects of Mutations on Side-Specific Folding Mechanism of a Helix-Turn-Helix Protein

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Helix-turn-helix motifs are important super-secondary protein structural elements and excellent models for studying the mechanism of protein folding. We have been investigating folding of a *de novo* designed 38-residue helix-turn-helix motif α-t-α using IR spectroscopy with site-specific ^{13}C isotopic editing. Our preliminary site-specific thermal unfolding data revealed that α-t-α is most stable near the centers of both α-helices, and likely unfolds from the helical termini and the loose turn region. To obtain further insights into the folding mechanism, and to investigate the roles of the individual residue-residue stabilizing interactions, we have begun mutational studies of the α-t-α protein. The mutations were designed to both destabilize and further stabilize the hydrophobic core near the helical centers. Additional mutations were designed to stabilize the helical termini and the turn/loop sequence. The overall thermodynamic stability of the α-t-α was measured using CD and IR spectroscopies. The core mutations appreciably decreased or increased the overall folding stability as intended, however, stabilizing the turn and helical termini proved to be a rather challenging task. Site-specific thermal unfolding of the mutated α-t-α were probed with IR on multiple ^{13}C isotopically labeled variants of each mutant. The effects of the mutations on both the global and, in particular, local site-specific unfolding provide important clues about the stabilization of the helix-turn-helix motif by specific interactions. Although additional mutational studies are underway, thus far all the data are consistent with the proposed folding mechanism.

167-Pos

Investigating Conformational Ensembles in Alanine Based Peptides Using Vibrational and Ecd Spectroscopy

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Short alanine based peptides are of interest to the protein community, due, in part to their departure from the statistical coil model. These peptides are too small to assume major secondary structures, but rather, have been found to adopt an ensemble of conformations in aqueous solution, with a predominance of PPII. However, experimental evidence suggests that the presence of charged residues might induce the sampling of multiple turn conformations, thus leading to a more compact structure of the peptide. To check this further, we measured the amide I profiles of the FTIR, Raman and VCD spectrum of H-(AAKAAW)-OH, and subsequently simulated the vibrational spectra using an excitonic coupling model, with NMR coupling constant and end-to-end distance constraints. We included multiple conformations: PPII, ß-strand, $_R$ helix, $_L$ helix, and turns. The alanine residues experienced a high propensity for PPII structure, ~70%, while ~20% for ß-strand conformations and smaller percentages for other coil structures. Lysine, however showed a larger propensity for ß-strand ~30% than the alanine residues, but the PPII content for lysine is still high (~42%). We obtained an end-to-end distance of 10Å, which is in accordance with FRET measurements of the end to end distance of